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Long-Lasting Spinal Oxytocin Analgesia Is Ensured by the Stimulation of Allopregnanolone Synthesis Which Potentiates $GABA_A$ Receptor-Mediated Synaptic Inhibition

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Hypothalamospinal control of spinal pain processing by oxytocin (OT) has received a lot of attention in recent years because of its potency to reduce pain symptoms in inflammatory and neuropathic conditions. However, cellular and molecular mechanisms underlying OT spinal antinociception are still poorly understood. In this study, we used biochemical, electrophysiological, and behavioral approaches to demonstrate that OT levels are elevated in the spinal cord of rats exhibiting pain symptoms, 24 h after the induction of inflammation with an intraplantar injection of λ-carrageenan. Using a selective OT receptor antagonist, we demonstrate that this elevated OT content is responsible for a tonic analgesia exerted on both mechanical and thermal modalities. This phenomenon appeared to be mediated by an OT receptor-mediated stimulation of neurosteroidogenesis, which leads to an increase in $GABA_A$ receptor-mediated synaptic inhibition in lamina II spinal cord neurons. We also provide evidence that this novel mechanism of OT-mediated spinal antinociception may be controlled by extracellular signal-related protein kinases, ERK1/2, after OT receptor activation. The oxytocinergic inhibitory control of spinal pain processing is emerging as an interesting target for future therapies since it recruits several molecular mechanisms, which are likely to exert a long-lasting analgesia through nongenomic and possibly genomic effects.

Introduction

Oxytocin (OT) is a nonapeptide synthetized by neurons located in the paraventricular (PVN) and supraoptic nuclei of the hypothalamus (Gimpl and Fahrenholz, 2001). If the neuroendocrine role of OT on the reproductive system is well known, its action as a neurotransmitter is yet to be understood. Oxytocinergic neurotransmission is ensured by parvocellular neurons of the PVN projecting to several regions of the CNS, including the spinal cord. In this particular region, there is a remarkable coexistence of OT-positive synaptic terminals (Saper et al., 1976; Swanson and McKellar, 1979; Sofroniew et al., 1981; Cechetto and Saper, 1988) and of OT binding sites in the most superficial layers of the dorsal horn and in autonomic nuclei (Reiter et al., 1994; Véronneau-Longueville et al., 1999). In good agreement with these anatomical data, OT seems to exert a potent antinociceptive action during spinal pain processing. Local application of OT or its endogenous release following PVN stimulation strongly reduces action potential firing of second-order neurons after recruitment of nociceptive-specific C and Aδ sensory neurons (Condés-Lara et al., 2003, 2006). OT-induced inhibition of nociceptive processing is further supported by the reduced glutamatergic transmission at sensorispinal synapses (Robinson et al., 2002), which contributes for a large part to short-lasting hyperexcitability (windup responses) and long-lasting plastic changes (long-term potentiation) in this preparation (Condés-Lara et al., 2006; DeLaTorre et al., 2009). Using patch-clamp recordings of lamina II neurons in spinal cord slices, we recently proposed OT action to be produced by a tonic synaptic release of GABA and by the inhibition of voltage-gated potassium currents responsible for repetitive firing in a subset of spinal neurons (Breton et al., 2008, 2009). These molecular mechanisms, and possibly others still unidentified, are likely to be recruited in inflammatory and neuropathic pain states since OT displayed significant analgesia in several rodent models (Lundeberg et al., 1993; Petersson et al., 2001; Yu et al., 2003; Condés-Lara et al., 2005; Miranda-Cardenas et al., 2006; Martínez-Lorenzana et al., 2008).

At this stage, results from our laboratory and other groups show that OT action on the excitability of superficial dorsal horn spinal neuron is potent, long-lasting, and particularly difficult to reverse (Breton et al., 2008, 2009; DeLaTorre et al., 2009). The major molecular target involved in OT-induced spinal antinociception is constituted by $GABA_A$ receptor channels ($GABA_A$Rs)

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but nothing is known on how OT can exert long-term antinociception and if OT can be tonically released by hypothalamospinal axons in pain conditions. To answer these questions, we used rats exhibiting inflammatory pain symptoms 24 h after an intraplantar injection of λ-carrageenan to measure the associated changes in spinal OT concentrations. Because these concentrations were elevated in hyperalgesic rats, we then characterized how these changes affect spinal nociceptive processing and the expression of pain symptoms. Eventually, a possible long-lasting action on $GABA_A$ receptor-mediated synaptic transmission was investigated.

Materials and Methods

Carrageenan model of pain

Male Sprague Dawley rats (9–11 weeks, 250–350 g; Janvier) were used for this part of the study. They were housed by group of four below standard conditions (room temperature, 22°C; 12 h light/dark cycle) with *ad libitum* access to food and water. All animals were habituated to the room and handled by the experimenter for at least 2 weeks. After this period, a single intraplantar injection (150 μl) of λ-carrageenan (3% in NaCl 0.9%; Sigma) was performed in the plantar surface of the right hindpaw for behavioral experiments whereas injections in both hindpaws were done for electrophysiological and biochemical studies. Control animals received an injection of NaCl 0.9% or no injection. All experiments were conducted in conformity with the directive of the European Committee Council Direction of September 22, 2010 (2010/63/EU) and with a protocol agreement given by French Department of Agriculture (License No. 67–116 to PP). Carrageenan model of inflammatory sensitization and the associated intrathecal procedures were submitted and approved by the regional ethic committee for animal experimentation (CREMEAS authorization numbers AL 11/14/03/07 and AL 04/13/05/08).

Oxytocin radioimmunoassay

Tissue preparation. The 9- to 11-week-old male Sprague Dawley rats (Janvier) were deeply anesthetized with a mixture of ketamine (75 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.). They were killed 24 h after the bilateral injection of λ-carrageenan (carrageenan group: $n = 5$) or saline injections (saline group: $n = 5$). Five control rats (not injected) of the same weight were added to the study. The spinal cord of each rat was collected by hydraulic extrusion and cut in slices 1 mm thick by a tissue chopper (McIlwain). To limit degradation of the peptides, slice cutting and sample preparation was performed on melting ice. Spinal cord slices, placed in tubes containing 60 μl of HCl (0.1 N), were sonicated for 30 s, and the resulting homogenate was centrifuged 15 min at 12,000 rpm. OT dosage was realized in 5 μl aliquot fractions of the supernatant.

OT assay. The principle of the assay is based on the competition between endogenous unlabeled OT and exogenously supplied iodine-125 radiolabeled OT (^{125}I-OT) for a specific antibody directed against OT (Moos et al., 1984). ^{125}I-OT was produced in the laboratory by redox reaction between OT and ^{125}I-Na, catalyzed by lactoperoxidase and hydrogen peroxide (Moos et al., 1984). The product of this reaction was purified by HPLC. For each sample, incubations were performed with 100 μl of spinal cord extract (unknown concentration of OT), 100 μl of antiserum (diluted to give 50% of iodinated hormone bound with the antibody in the absence of antigen), and 100 μl of ^{125}I-OT (1000 counts/min per tube). All dilutions were made in a barbital buffer (0.05 M sodium barbital, pH 8.6, 2.5 mg/l bovine serum albumin, and 0.02% sodium merthiolate). After a 6 d incubation at 4°C, bound and free hormone were separated by charcoal adsorption and then by adding 100 μl of bovine serum and 1 ml of charcoal-dextran suspension (Charcoal Norit C 170, Fisher Scientific; Dextran T70, Pharmacia). Suspension was centrifuged at 800 rpm for 20 min at 4°C and the supernatant containing the OT-antibody complexes was discarded; the ^{125}I-OT retained by the charcoal suspension was quantified by a gamma counter (Packard) and compared with standard displacement curves obtained with known concentration of OT. OT content is expressed in pg/mm of spinal cord and the vertebral level was identified before cutting.

FosB/ΔFosB immunohistochemistry

Immunohistochemistry was performed on a separate set of animals ($n = 6$ per group). One day following intraplantar injection of either carrageenan or saline solution, rats were perfused intracardially with 150 ml of PB (0.1 M, pH 7.4) followed by 500 ml of a solution containing 4% paraformaldehyde in PB. After laminectomy, the lumbar spinal cord (L3–L5) segments were collected, immersed overnight in the same fixative, and washed the next day in phosphate saline buffer (PBS). Transverse sections of 40 μm were prepared using a tissue slicer (Leica VT1000S). The sections were rinsed three times during 10 min in PBS and subsequently incubated for 1 h in a blocking solution composed of 5% donkey serum in PBS and 0.5% Triton X-100. The sections were then incubated overnight with a "pan-FosB" antibody (rabbit polyclonal 1:1000, ref. sc-48; Santa Cruz Biotechnology) raised against an internal region of FosB (i.e., recognizing both FosB and ΔFosB proteins) and with neurophysin-OT (1:250; kindly provided by Dr. Pierre Veinante, University of Strasbourg, Strasbourg, France) diluted in PBS containing 1% normal serum and 0.5% Triton X-100. After washing with PBS (5 × 10 min), the sections were incubated 90 min with the secondary antibodies (Cy3:1:800, FITC: 1:80; Sigma-Aldrich) for 2 h at room temperature. After rinsing with PBS (3 × 10 min), sections were mounted on gelatin-coated slides. Three sections per animal were randomly chosen and the number of activated neurons (FosB) and of OT-positive cells (neurophysin-OT) in the paraventricular nucleus was assessed.

Electrophysiological recordings

Single-unit extracellular recording of dorsal horn spinal neurons in vivo. The 9- to 11-week-old Sprague Dawley rats were anesthetized with 3% isoflurane in pure oxygen and a laminectomy was performed to expose the L4–L5 spinal cord segments. The animal was then placed on a stereotaxic frame, with the L4–L5 exposed region being firmly held by two clamps placed on the apophysis of the rostral and caudal intact vertebras. Isoflurane anesthesia was then reduced to 1.5% and a stainless steel electrode (10 MΩ; FHC) was lowered in the dorsal horn of the spinal cord after meninges removal. Single-unit extracellular recordings were all made from deep dorsal horn neurons, located 400–800 μm from the dorsal surface (see Fig. 1*B*), receiving convergent (non-noxious and noxious) sensory information from the ipsilateral hindpaw as previously described (Juif and Poisbeau, 2013). Voltage changes were amplified and filtered (low: 300 Hz; high: 3000 Hz) by a DAM-80 differential amplifier (WPI). Recordings were acquired at 10 kHz and analyzed using the Spike 2 software, collecting data through a CED 1401 analog-to-digital interface (Cambridge Electronic Design). The stimulating threshold to evoke action potentials and their frequency of occurrence, resulting from the stimulation of the peripheral receptive field on the rat hindpaw, were attributed to the recruitment of A- and C-type sensory fibers based on their latency from the stimulus artifact as follows: Aβ: <20 ms; Aδ: 20–90 ms; C: 90–300 ms; post discharge: 300–800 ms. Modulation of spinal cord neuron excitability was determined on action potential firing responses induced by electrical stimuli of the hindpaw (1 ms pulse duration, frequency 0.2 Hz), at an intensity corresponding to 1.5 times the threshold for C-type sensory fiber activation.

Patch-clamp recording of lamina II neurons in spinal cord slices. Transverse slices of lumbar spinal cord were prepared from young adult Sprague Dawley rats (P21–P55; Janvier) deeply anesthetized with a mixture of ketamine (75 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.), as described in previous studies (Keller et al., 2001). Although animals were younger than those used for the OT dosage, pain behavior, and *in vivo* electrophysiology, we found no difference in the synaptic current characteristics while recording from neurons in spinal cord slices of P21 rats and older animals (maximum age P55). This observation was in agreement with our previous observation (Keller et al., 2001) and data were pooled. Animals were killed 24 h after having performed the carrageenan or saline (bilateral) hindpaw injections. The lumbar spinal cord was collected by hydraulic extrusion and placed into ice-cold sucrose artificial CSF (S-ACSF) containing the following (in mM): 248 sucrose, 11 glucose, 23 $NaHCO_3$, 2 KCl, 1.25 KH_2PO_4, 2 $CaCl_2$, and 1.3 $MgSO_4$ and was continuously saturated with 95% O_2–5% CO_2. Transverse slices (300 μm thick) were cut from the lumbar segment of the spinal cord using a

vibratome and stored at room temperature in an incubation chamber filled with regular ACSF containing the following (in mM): 126 NaCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 2.5 KCl, 2 $CaCl_2$, 2 $MgCl_2$, and 10 glucose and continuously saturated with 95% O_2–5% CO_2. Spinal cord slices were stored for at least 1 h at room temperature before being placed into the recording chamber. The slices were continuously superfused with oxygenated ACSF at a flow rate of ~3 ml/min.

All electrophysiological experiments were performed at room temperature in regular ACSF and lamina II neurons were recorded using the whole-cell configuration of the patch-clamp technique. The patch pipette was filled with a solution containing the following (in mM): 125 CsCl, 2 $MgCl_2$, 2 Mg-ATP, 0.2 Na-GTP, and 10 HEPES, pH, 7.3 adjusted with CsOH. Neurons were voltage clamped at −60 mV using an Axopatch 200B amplifier (Molecular Devices); series capacitance (Cs) and resistance were (Rs) compensated electronically throughout the experiments and only recordings lasting longer than 20 min with series resistance <20 MΩ were considered for analysis. All recordings were low-pass filtered at 5 kHz, digitized at 10 kHz, and stored on computer using the Clampex module of pClamp software (Molecular Devices) before being analyzed. $GABA_A$ receptor-mediated miniature IPSCs ($GABA_AR$ mIPSCs) were pharmacologically isolated by using an ACSF containing 0.5 μM tetrodotoxin (Latoxan), 2 mM kynurenic acid (Fluka), and 1 μM strychnine (Sigma). Off-line, $GABA_AR$ mIPSCs were detected and analyzed individually using WinEDR and WinWCP software (courtesy of Dr. J. Dempster, University of Strathclyde, Glasgow, UK) to examine their frequency of occurrence, amplitude, and monoexponential decay time constants (τ).

Allopregnanolone dosage

Transverse slices of lumbar spinal cord were prepared from Sprague Dawley rats (between 30 and 50 d old; Janvier) anesthetized with a mixture of ketamine (75 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.). Slices (1 mm length) were then placed for 3 h at room temperature in an incubation chamber filled with regular ACSF (see above) supplied with either [Thr^4,Gly^7]-oxytocin (TGOT; 1 μM) or TGOT (1 μM) + d(CH_2)$_5$-[$Tyr(Me)^2$,Thr^4,Orn^8,des-Gly-NH_2^9-vasotocin (dOVT; 1 μM) continuously saturated with 95% O_2–5% CO_2. After this incubation period, sections were homogenized in 200 μl of ultrapure water and 600 μl of methanol (100%) were added. Samples were centrifuged during 30 min at 14,000 × *g* (4°C). Then, supernatant was recovered and dried with a SpeedVac evaporator. Sample extracts were dissolved in 200 μl of ELISA sample buffer before quantification. An allopregnanolone-ELISA kit (GENTAUR) was used to quantify allopregnanolone concentrations in spinal cord extracts (50 μl in duplicate) following the manufacturer's instructions. For all tests, allopregnanolone standards were diluted in the sample buffer to avoid matrix effects. The coefficient of variation (CV) values were between 0 and 8%. All samples with a higher CV value were tested again to obtain a CV below or equal to 8%. The calculated methodological detection limit of the batch of ELISA kit used for the study was 1 ng/ml of allopregnanolone. Amounts of allopregnanolone (ng) were normalized according to protein's concentrations (Protein Assay; Bio-Rad) determined before the extraction procedure. Note that cross reactivity of the antibody is ~15% for progesterone and 17-hydroxyprogesterone (source: manufacturer) and 10.9% for allotetrahydrodeoxycorticosterone (present study).

Behavioral testing

Habituated adult Sprague Dawley rats ($n = 6$ per group) were used to measure nociceptive thresholds while applying mechanical and thermal heat stimuli on their hindpaws. All behavioral tests were done during the light period (between 9:00 and 16:00). Mechanical sensitivity was measured using a calibrated forceps (Bioseb) previously validated in our laboratory (Luis-Delgado et al., 2006). Briefly, the rat was loosely restrained with a towel masking the eyes to limit stress by environmental stimulation. The tips of the forceps were placed on each side of the hindpaw and a gradually increasing force was applied. The pressure producing paw withdrawal indicated the nociceptive threshold value. The thermal heat nociceptive response was assessed using Hargreaves' test (Bioseb), allowing the differentiation of the response of each hindpaw (Hargreaves et al., 1988). Hindpaws are exposed to a radiant heat and the latency time required to induce paw withdrawal from the surface is taken as the nociceptive heat threshold. Nociceptive pressure threshold or withdrawal latency measurements were performed three times for each hindpaw and the values were averaged.

Drugs and treatments

We used the selective OT receptor agonist TGOT (Sigma) and antagonist dOVT (Bachem). OT receptors ligands were prepared as ×1000 stock solutions in a molar acetic acid solution 0.25% (v/v) in distilled water. These drugs were then added to the ACSF for *in vitro* electrophysiological experiments and to saline solution for behavioral experiments and *in vivo* electrophysiological recordings. The vehicle, used to prepare stock solutions and diluted at least 1000 times, had no electrophysiological or behavioral effects in the present study. To block neurosteroidogenesis *in vitro*, we incubated spinal cord slices >3 h in oxygenated ACSF containing PK11195 [1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinoline carboxamide: an inhibitor of the cholesterol translocator protein complex (TSPO; Steraloids)] and finasteride [1,(5α)-androsten-4-aza-3-one-17β-(*N*-*tert*-butyl-carboxamide): an inhibitor of type II 5α-reductase (Steraloids)]. In such experiments, the control condition consisted of adjacent slices incubated under the same conditions but in the absence of the pharmacological agents. *In vivo*, rats received a subcutaneous injection (scapular region), every 2 d, of finasteride (FIN; 30 mg/kg) or Depo-Provera (4-pregnen-6α-methyl-17-ol-3, 20-dione acetate: inhibitor of 3α-hydroxysteroid dehydrogenase; 5 mg/kg; Steraloids) prepared in an olive oil vehicle with 20% v/v ethanol. Treatment was administered for at least 1 week before λ-carrageenan injection and until the end of the behavioral testing. To inhibit the activity of ERK1/2 *in vitro*, we incubated spinal cord slices in ACSF containing 20 μM PD09859 (Merck-Millipore). With an IC_{50} of 2 μM, an inhibition of ~90% of the ERK1/2 activity is likely to be achieved while using a steady-state concentration of 20 μM (Alessi et al., 1995).

Intrathecal injections

Oxytocin receptor agonist (TGOT) and antagonist (dOVT) were administered intrathecally on rats, anesthetized with halothane 3% (in pure oxygen). Acute intrathecal injection was realized by puncture through the intervertebral space between L5 and L6 using a 50 μl Hamilton microsyringe with a 26 gauge needle. A characteristic tail flick indicated penetration of the needle in the intrathecal space and ensures success for the delivery of the drug. After injection of the drug solution or of the vehicle (NaCl 9‰; 10 μl), the animal immediately recovered from halothane anesthesia and was placed in the testing chamber. To estimate possible spinal effect of the neurosteroids, rats were tested for mechanical thermal nociception 1, 4, and 24 h after the intrathecal injection, to check for recovery.

Statistical analysis

All data are expressed as mean ± SEM. One-way or two-way ANOVA were used to analyze the effects of treatments (between factors), of side (between factors), and of time (within factor). Significant differences were assessed using Tukey's or Bonferroni *post hoc* tests (Statistica; StatSoft). Differences were considered to be statistically significant for $p < 0.05$. Kolmogorov–Smirnov test (KS test) was used to compare cumulative distributions and they were considered different when $p < 0.01$. Two-tailed paired and unpaired Student's *t* tests and nonparametric Mann–Whitney test were also used to compare two or more groups. Differences were considered to be statistically significant for $p < 0.05$.

Results

Increased spinal OT content exerts a tonic inhibitory control of spinal nociceptive processing in carrageenan-injected animals

OT content from rat spinal cord was quantified by radioimmunoassay (RIA) 24 h after a bilateral intraplantar injection of λ-carrageenan and compared with the control groups: vehicle-injected (saline) and not injected (naive). In naive ($n = 5$ rats) and saline-injected rats ($n = 5$), OT content was similar for all

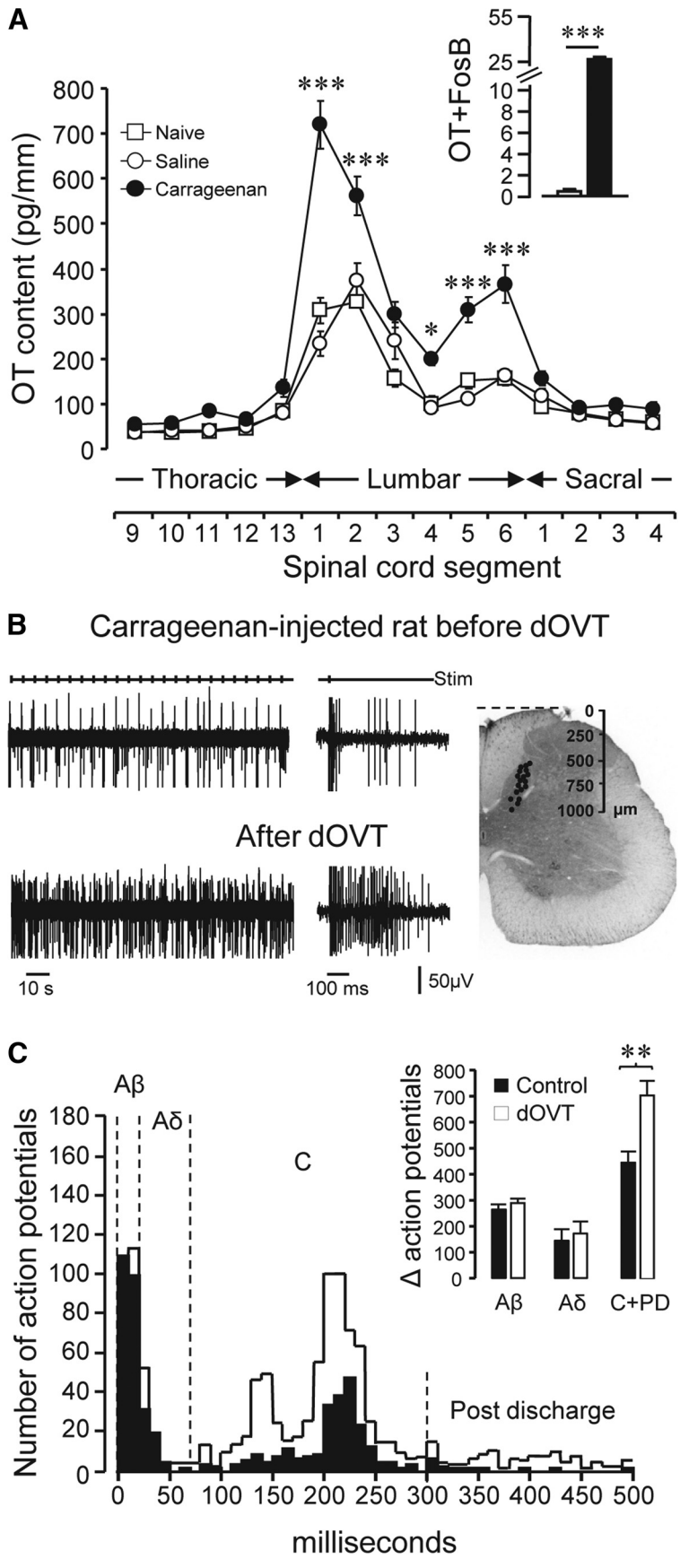

Figure 1. Elevated lumbar content in OT accounts for a limitation of spinal nociceptive processing in the carrageenan model of inflammatory pain sensitization. ***A***, OT content measured in thoracolumbar spinal vertebral segments (from T9 to S4) of naive (not injected, open squares), saline-injected (open circles), and carrageenan-injected (filled circles) animals. Twenty-four hours after the injection, carrageenan animals had significantly higher OT content in L1, L2, L5, and L6 vertebral segments compared with naive and saline-injected animals. (Asterisks indicate statistical difference after Tukey's *post hoc* test within each spinal cord segment.) Inset, Histogram illustrating the number of OT-neurophysin-positive neurons in the PVN also labeled for FosB/ΔFosB in control (saline: white bar) and carrageenan-injected rats (black bar). ***B***, Representative *in vivo* recording from a carrageenan-injected rat (24 h after the injection) showing typical evoked action potential responses of a deep dorsal horn neuron located in the lumbar enlargement, after the stimulation of the corresponding ipsilateral hindpaw receptive field. Note that local application of the OT receptor antagonist dOVT (1 μM) induced a strong increase in firing frequency (see expended traces in the middle). On the right is indicated the location of recorded neurons. ***C***, Histogram illustrating changes in the number of action potentials per period of 10 min (stimulation at 0.2 Hz), before and after application of dOVT, for the neuron illustrated in ***B***. For all recorded neurons ($n = 8$), the mean number of action potentials per PAF type [Aβ, Aδ, C, and post discharge (PD)] is also illustrated in the inset. Statistical difference was assessed between groups using two-tailed paired Student's *t* test. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

spinal cord vertebral segments and was <70 pg/mm of spinal cord. However, spinal OT content peaked at thoracic levels T2–T4 (Control: 103.3 ± 5.8 pg/mm; Saline: 98.8 ± 7.7 pg/mm; data not shown) and at lumbar levels L1–L2 and L5–L6 (Fig. 1*A*). In carrageenan-injected rats ($n = 5$), OT content was similar to the corresponding level of control and saline-injected groups but concentrations were significantly higher at most lumbar segments (L1–L2, L4–L6) with the Tukey *post hoc* test (Fig. 1*A*) after having performed an ANOVA between the different groups (two-way ANOVA, interaction between treatment and spinal cord segment: $F_{(28,180)} = 13.59$, $p < 0.001$).

Because oxytocinergic neurons, projecting to the spinal cord, are mostly located in the paraventricular nuclei, we next verified if the observed release of OT following carrageenan-induced inflammation resulted from the activation of these neurons. This was done by quantifying the number of activated oxytocinergic neurons using a double staining for neurophysin-OT and FosB/ΔFosB after carrageenan or saline intraplantar injection (Fig. 1*A*, inset). As expected, the total number of FosB/ΔFosB-immunoreactive cells in the PVN was significantly increased in carrageenan-treated animals (41.7 ± 2.0, $n = 4$) compared with noninflamed animals (1.6 ± 0.5, $n = 4$; Mann–Whitney, $p < 0.001$). Interestingly, this was also true while counting neurophysin-OT-immunopositive neurons (control: 9.0 ± 1.0; carrageenan: 65.9 ± 4.6; Mann–Whitney, $p <$

Table 1. Action potential firing of WDR neurons recorded from the spinal cord of carrageenan-induced hyperalgesic rats

Treatment	Aβ	Aδ	C	Post discharge	N
Control	264 ± 20	145 ± 45	486 ± 106	248 ± 91	8
dOVT, intrathecal.	289 ± 17	179 ± 46	693 ± 108**	361 ± 95	8

Deep dorsal horn neuron action potential responses to electrical stimulation of the hindpaw (see Materials and Methods) are classified based on their latency to the stimulation as follows: Aβ: < 20 ms; Aδ: 20–90 ms; C: 90–300 ms; post discharge: 300–800 ms. Results were obtained from carrageenan-injected rats before (Control) and after local spinal dOVT application. Values are indicated as mean ± SEM Statistical significance was assessed by comparing the groups before and after dOVT using two-tailed paired Student's *t* test (**$p < 0.01$).

0.001) fully in agreement with our RIA data. We observed that the number of colocalized OT/FosB neurons (Fig. 1*A*, inset) was significantly higher in carrageenan-injected rats (26.7 ± 1.5, $n = 4$) compared with control animals (0.4 ± 0.2, $n = 4$; Mann–Whitney, $p < 0.001$). We next answered the question of what could be the functional consequences of the elevated OT spinal content on spinal nociceptive processing and $GABA_A$R-mediated inhibitory synaptic transmission.

We first recorded dorsal horn neuron responses after the electrical stimulation of the hindpaw receptive field. We choose to record wide dynamic range neurons (WDR; Fig. 1*B*), which integrate convergent peripheral sensory information from non-nociceptive Aβ type and nociceptive Aδ and C-type primary afferent fibers (PAFs). Local spinal application of an OT receptor-antagonist, dOVT, was performed to reveal a possible effect of endogenous OT on inhibitory tone in saline- and carrageenan-injected rat. In saline-injected animals (and also in naive; data not shown), local lumbar dOVT application (1 μM) had no obvious effect on deep dorsal horn neurons excitability in response to the activation of fast and slow conducting PAFs (number of action potentials as well as PAF thresholds were unchanged; data not shown, $n = 6$). In contrast, C-type PAF threshold was significantly decreased in carrageenan-injected rats (1.38 ± 0.10 mA, $n = 8$) compared with the control group (2.11 ± 0.71 mA, $n = 8$; unpaired Student's *t* test: $t_{(14)} = 1.02$, $p < 0.05$), and this threshold was further decreased to 0.91 ± 0.06 mA ($n = 8$) after topical application of dOVT on the spinal cord compared with value before dOVT (paired Student's *t* test: $t_{(7)} = 5.95$, $p < 0.001$). In addition, a train of 120 stimulations (frequency: 0.2 Hz, intensity 1.5 times the C-type fiber threshold; pulse duration: 1 ms) was used to quantify the number of action potentials emitted by the recorded neuron. As illustrated in Figure 1, *B* and *C*, a significant increase in the number of action potentials, resulting from the stimulation of C-type PAFs (post discharge included), was observed in carrageenan-injected rats after spinal application of dOVT (before dOVT: number of action potentials = 444 ± 42; after dOVT: number of action potentials = 703 ± 56; $n = 8$; $t_{(7)} = 6.621$ paired Student's *t* test: $p < 0.01$), whereas the response to A-type PAF activation was not affected during spinal application of dOVT (Fig. 1*C*; see also Table 1). In a subset of experiment we further demonstrated that 25% of the recorded WDR neurons ($n = 20$) were projecting neurons since they displayed a retrograde action potential after stimulation of the anterolateral spinal quadrant (intensity: 200 μA, pulse duration: 100 μs), a few millimeters rostrally to the recording site. All together, these results show that peripheral inflammation induced by injection of carrageenan in the hindpaw increases OT content at the spinal lumbar level and this release of OT exerts a tonic inhibition of nociceptive C-type PAFs converging directly or indirectly on WDR neurons. Since all WDR neurons were submitted to this inhibitory action of OT (i.e., including projection neurons), we suggest here that supraspinal transmission of peripheral nociceptive messages will be reduced.

Long-term activation of OT receptor potentiates fast GABAergic synaptic transmission

To better understand how spinal OT could exert long-lasting antinociceptive effects in the neuronal networks of the spinal cord, we next incubated spinal cord slices from naive rats for at least 3 h at room temperature in regular ACSF or in ACSF containing 100 nM TGOT, a selective OT receptor agonist. Then, $GABA_A$R mIPSCs were recorded from neurons in lamina II, which represent a major target for nociceptive C-type PAFs. Amplitude, frequency of occurrence, rise time, and decay time constant of $GABA_A$R mIPSCs were analyzed (see Materials and Methods). Compared with the control condition, $GABA_A$R mIPSCs recorded after TGOT incubation differed only by their decaying kinetics (Fig. 2*A*,*D*, Table 2; one-way ANOVA: $F_{(4,34)} = 15.36$, $p < 0.001$). The mean monoexponential decay time constant (τ) was significantly slower compared with control (control: 20.4 ± 1.5 ms, $n = 8$; TGOT: 32.5 ± 2.3 ms, $n = 9$; Tukey *post hoc* test: $p < 0.001$). Interestingly, prolonged mIPSCs were never observed when spinal cord slices were incubated in the presence of TGOT (100 nM) and dOVT (1 μM; Table 2, Fig. 2*A*,*D*; Tukey *post hoc* test: $p > 0.05$). All together, these results strongly support the idea that endogenously released OT exerts a tonic inhibitory control on lamina II neurons mediated by a potentiation of $GABA_A$R-mediated synaptic tone.

OT long-lasting potentiation of $GABA_A$ mIPSCs is achieved by the stimulation of allopregnanolone synthesis: possible role of extracellular signal-regulated kinases

Our laboratory has provided evidence indicating that endogenous neurosteroids are produced in the spinal cord after peripheral inflammation induced by intraplantar injection of carrageenan. In particular, the spinal synthesis of 3α5α-reduced neurosteroids limits the intensity of pain symptoms by potentiating GABAergic transmission in lamina II interneurons (Poisbeau et al., 2005). In good agreement with our previous findings and compared with control (saline injected), $GABA_A$R mIPSCs recorded from lamina II spinal cord neurons of hyperalgesic rats (24 h after carrageenan intraplantar injection) exhibited a significant prolonged decaying phase (control: $\tau = 20.4 \pm 1.2$ ms, $n = 9$; carrageenan: $\tau = 30.7 \pm 1.2$ ms, $n = 12$; one-way ANOVA: $F_{(4,46)} = 25.06$, $p < 0.001$; Tukey *post hoc* test: $p < 0.001$; Fig. 2*D*). No differences were noted for the other parameters, i.e., mean amplitude, rise time, and frequency of occurrence (data not shown). Interestingly, $GABA_A$R mIPSCs recorded from lamina II neurons during hyperalgesia were similar to those recorded from TGOT-incubated lamina II neurons of naive rats (Fig. 2*D*; TGOT incubation: $\tau = 32.5 \pm 2.3$ ms, $n = 9$; carrageenan injection: $\tau = 30.7 \pm 1.2$ ms, $n = 12$). Furthermore, inhibition of 3α5α-reduced neurosteroid synthesis by subcutaneous injection of finasteride (FIN: 5α-reductase inhibitor; 30 mg/kg every 2 d; Fig. 2*B–D*), 1 week before the injection of carrageenan in the hindpaw, fully occluded the increase in $GABA_A$R mIPSC decays, which were similar to the control group (Tukey *post hoc* test: $p > 0.001$; Fig. 2*B*,*D*).

To reveal a possible oxytocinergic control of neurosteroidogenesis, we next incubated spinal cord slices of naive rats for 3 h in ACSF containing TGOT (100 nM) and two inhibitors of neurosteroidogenesis (Fig. 2*C*): the 5α-reductase inhibitor finasteride (50 μM) and the mitochondrial TSPO inhibitor PK11195 (10

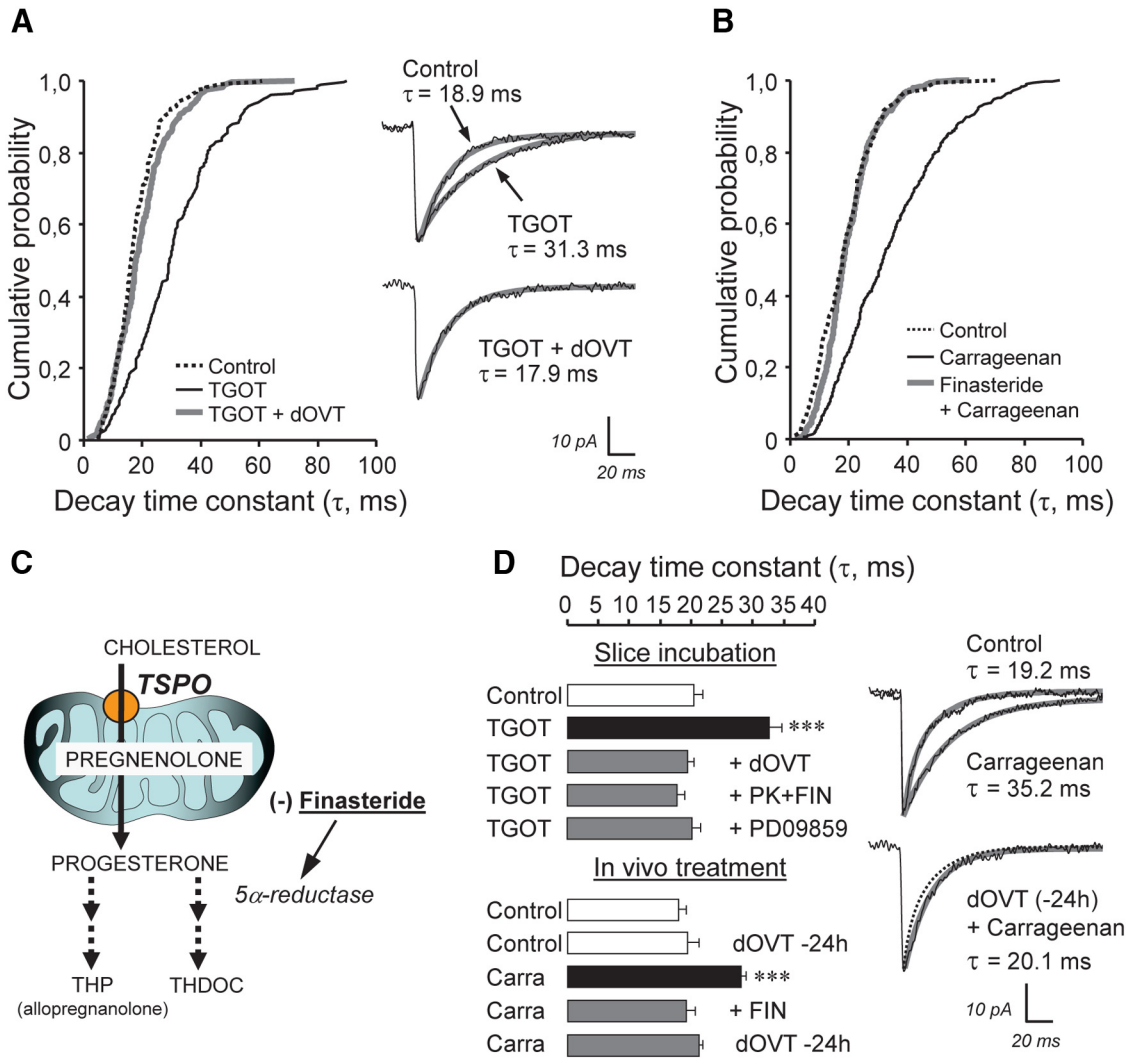

Figure 2. OT receptor activation increases the inhibitory charge carried by synaptic $GABA_A$Rs in lamina II neurons through the stimulation of 3α5α neurosteroids synthesis. ***A***, ***B***, Cumulative probability graphs of $GABA_A$ mIPSC decay time constants (τ) recorded in lamina II neurons of rat spinal cord slice (***A***) incubated 3 h in control ACSF (Naive: dotted line, $n = 281$ events), TGOT (100 nM: thin black line, $n = 200$ events), or TGOT and dOVT (1 μM; thick gray line, $n = 207$ events) and (***B***) of control (not injected: dotted line, $n = 117$ events) and carrageenan-injected animals, pretreated with finasteride (thick gray line, $n = 616$ events) or not (thin black line, $n = 414$ events). Traces in ***A*** are an average of 10 individual $GABA_A$R mIPSCs in the different experimental conditions. Note that neurons incubated in TGOT exhibited significantly prolonged mIPSC decay time constant (compared with control, KS test, $p < 0.001$) and this effect was occluded in slices, incubated in the presence of TGOT and dOVT (KS test, $p > 0.05$). Decay time constant of $GABA_A$R mIPSCs recorded in the carrageenan condition were also slower compared with those of control animals (KS test, $p < 0.001$) and of finasteride-pretreated carrageenan (KS test, $p < 0.001$). ***C***, Simplified schematic showing the metabolic pathway that leads to the production of 3α5α neurosteroids: allopregnanolone (THP) and tetrahydrodeoxycorticosterone (THDOC), two potent positive allosteric modulators of $GABA_A$ receptor function. Finasteride and PK11195 were used to inhibit 5α-reductase activity and TSPO function, respectively. ***D***, Histogram comparing the mean decay time constants of $GABA_A$R mIPSCs recorded from lamina II neurons in various experimental conditions: (top graph) *in vitro* slice incubation (3 h) with normal ACSF (control), TGOT alone (100 nM), or in combination with dOVT (1 μM), PK11195 + Finasteride (PK + FIN), or PD09859 (20 μM); (bottom graph) *in vivo* pretreatment of animals with finasteride (30 mg/kg, s.c) for 7 d or dOVT (10 ng, intrathecally) 24 h before carrageenan sensitization (Carra) are compared with a saline-injected group (control). Traces on the right are an average of 10 individual $GABA_A$R mIPSCs recorded from control, carrageenan, and dOVT + carrageenan-treated rats. One-way ANOVAs performed to compare treatments for *in vitro* and *in vivo* conditions were significantly different. Tukey's *post hoc* test indicates significance at $^{***}p < 0.001$.

μM). Inhibition of spinal neurosteroidogenesis *in vitro* occluded the effect of TGOT incubation on the decay of $GABA_A$R mIPSCs (Table 2, Fig. 2*B*,*D*; one-way ANOVA: $F_{(4,34)} = 15.36$, $p < 0.001$; Tukey *post hoc* test: $p > 0.05$). This result strongly suggested that spinal OT receptor activation is required for the potentiation of $GABA_A$R mIPSCs by locally produced 3α5α-reduced neurosteroids. This conclusion was further supported by the quantification of allopregnanolone in lumbar spinal cord sections incubated for 3 h in TGOT alone or TGOT + dOVT. Allopregnanolone levels were 0.11 ± 0.04 ng/mg protein ($n = 10$) after TGOT incubation but significantly lower and barely detectable after TGOT + dOVT (0.02 ± 0.03 ng/mg protein, $n = 10$; Mann–Whitney, $p < 0.01$).

Although OT receptor-mediated intracellular signaling cascade is still unclear in the CNS, evidence suggests that ERKs are likely to constitute a converging pathway. We examined this hypothesis by analyzing the decaying kinetics of $GABA_A$R mIPSCs after a 3 h coincubation of spinal cord slices in the presence of TGOT (1 μM) and of the MEK pathway inhibitor PD09859 (20 μM). Under this experimental condition, the resulting mean decay time constant was of 20.6 ± 0.9 ms ($n = 7$) and similar to control slices (incubated in regular ACSF) taken from naive rats ($p > 0.05$ Tukey *post hoc* test). This result likely indicated that ERK1/2 was indeed recruited after OT receptor activation and could account for the stimulation of neurosteroidogenesis and the subsequent tonic potentiation of $GABA_A$R-mediated synap-

Table 2. OT receptor activation potentiates $GABA_A$ receptor-mediated mIPSCs in lamina II neurons of the spinal cord

mIPSC parameters	Amplitude (pA)	Decay (τ)	Frequency (Hz)	*N*
Control incubation	30.8 ± 3.4	20.4 ± 1.5	0.305 ± 0.047	8
TGOT	33.1 ± 2.5	32.5 ± 2.3*	0.259 ± 0.036	9
TGOT + dOVT	33.0 ± 1.2	19.4 ± 1.1	0.322 ± 0.046	7
dOVT incubation	32.5 ± 1.0	21.3 ± 0.5	0.321 ± 0.030	10
TGOT + PK11195 + finasteride	35.6 ± 1.2	17.7 ± 1.2	0.444 ± 0.076	8

Characteristics of $GABA_A$R mIPSCs recorded from lamina II neurons of rat spinal cord after a 3 h incubation in the following solutions: control (regular ACSF), TGOT (100 nM), TGOT (100 nM) + dOVT (1 μM), dOVT (1 μM), and TGOT (100 nM) + PK11195 (10 μM) + finasteride (50 μM). Values are indicated as mean ± SEM Statistical significance between treatments was observed by one-way ANOVA only in the case of decay. Tukey's *post hoc* tests indicated a statistical difference for decay time constants of mIPSCs recorded from the TGOT-incubated spinal cord slices (*$p < 0.05$).

tic transmission in lamina II spinal cord neurons. This preliminary result, while interesting, will need to be consolidated by additional investigations to fully dissect the underlying signaling cascade, which may involve other well known kinases.

To further strengthen this conclusion, dOVT was injected intrathecally in the lumbar segment of the rat spinal cord (10 ng; volume: 10 μl) 24 h before the intraplantar carrageenan injection. Spinal cord slices (of the lumbar enlargement) from these rats were then prepared, 48 h after dOVT intrathecal injection and 24 h after carrageenan intraplantar injection, to record $GABA_A$R mIPSCs from lamina II neurons. In good agreement with our *in vitro* incubation result, lamina II neurons recorded from dOVT-pretreated and carrageenan-injected rats exhibited $GABA_A$R mIPSCs similar to those recorded from the control group. In particular, we failed to reveal any differences in the mean decay time constant ($\tau = 20.6 \pm 0.9$ ms; $n = 12$; $p > 0.05$ Tukey *post hoc* test; Fig. 2*D*). Furthermore, intrathecal injection of dOVT (alone) in naive animals was not associated with any change in the decay time constant of $GABA_A$R mIPSCs (Fig. 2*D*; dOVT −24 h: $\tau = 19.3 \pm 1.0$ ms, $n = 9$; compared with control, $p > 0.05$ Tukey *post hoc* test). All together, our results provide convincing evidence that a tonic activation of spinal OT receptors modulates $GABA_A$ inhibitory tone in carrageenan-induced pain animals in lamina II neurons. This mechanism is ensured by a stimulated synthesis of 3α5α-reduced neurosteroids, which is ERK1/2 dependent. We next attempted to demonstrate that this endogenous mechanism could be responsible for the reduction of pain symptoms in carrageenan-induced hyperalgesic animals.

Tonic activation of spinal OT receptor stimulates the synthesis of 3α5α-reduced neurosteroids and limits mechanical and thermal heat hyperalgesia

Figure 3 illustrates the time course of mechanical (*A1*) and thermal hot hyperalgesia (*B1*) measured after a unilateral intraplantar carrageenan injection (right paw, black symbols). While mechanical and thermal nociceptive thresholds of the noninjected paw remained similar to the baseline values, mechanical and thermal hyperalgesia develop rapidly for the carrageenan-injected paw and reached its maximum 7 h after the injection (mechanical pressure threshold decrease from 333.8 ± 4.9 g before the injection to 19.5 ± 2.2 g; two-way ANOVA: time × treatment, $F_{(13,130)} = 76.45$, $p < 0.001$; Bonferroni: $p < 0.001$; thermal latency threshold decrease from 11.9 ± 0.5 s before the injection to 2.5 ± 0.5 s; $n = 6$ rats per group; two-way ANOVA: time × treatment, $F_{(9,81)} = 10.04$, $p < 0.001$; Bonferroni: $p < 0.001$; Fig. 3*A1–B1*). As indicated on the time course, and especially 24 h after carrageenan injection, rats remained hyperalgesic for mechanical (~43%) and thermal hot modalities (~54%).

We next characterized the effects of intrathecal injections of saline, TGOT (100 nM), and dOVT (1 μM) on mechanical and thermal nociceptive thresholds, 24 h after intraplantar carrageenan injection. This experiment was designed to demonstrate if endogenous OT tonus limits the intensity of pain symptom (dOVT intrathecal application) and if this OT-mediated antinociceptive control could be further potentiated (TGOT intrathecal application). Furthermore, TGOT and dOVT were administered to hyperalgesic rats, pretreated for at least 1 week with neurosteroidogenesis inhibitors (finasteride or Provera, see Materials and Methods) before the intraplantar carrageenan injection, to confirm or not that OT spinal analgesia requires the synthesis of 3α5α neurosteroids. It has to be noticed that the finasteride or Provera treatment did not produce any significant changes in the nociceptive thresholds per se. In the control group of rats (intact neurosteroidogenesis), intrathecal injection of TGOT (100 nM) had no significant effect on mechanical and thermal nociceptive threshold/latency (Fig. 3*A2–B2*, left graphs). In contrast, intrathecal injection of dOVT (1 μM) was associated with an immediate and significant decrease in mechanical threshold (from 205.6 ± 23.3 g to 81.8 ± 3.7 g; $n = 6$; two-way ANOVA: time × treatment, $F_{(4,30)} = 7.97$, $p < 0.001$; Bonferroni: $p < 0.001$; Fig. 3*A2*, left graph) and thermal hot latency (from 5.8 ± 0.4 s to 3.2 ± 0.5 s; $n = 6$; two-way ANOVA: time × treatment, $F_{(4,30)} = 8.13$, $p < 0.001$; Bonferroni: $p < 0.001$; Fig. 3*B2*, left graph), 4 h after intrathecal injection. As indicated in the Fig. 3, *A2* and *B2*, exacerbation of mechanical and thermal hyperalgesia following dOVT intrathecal injection was never observed in finasteride- or Provera-pretreated rats. Our *in vivo* results strongly suggest that the tonic activation spinal OT receptors in hyperalgesic rats leads to the local production of 3α-reduced neurosteroids, which limit the intensity of their pain symptoms. Potentiation of $GABA_A$ receptors in spinal nociceptive circuits are, for sure, a privileged target for this endogenous inhibitory control.

Discussion

Among the very few homeostatic analgesic mechanisms that have been identified so far in the CNS, cellular mechanisms leading to an efficient long-lasting antinociception are largely unknown. In this study, we have attempted to understand how the activation of descending hypothalamospinal oxytocinergic axons could exert such a tonic inhibitory control in the spinal cord, in a rat model of inflammatory sensitization following intraplantar carrageenan injection. Using different experimental approaches, biochemical, electrophysiological, and behavioral techniques, we show that the proinflammatory action of carrageenan in the paw is associated with the activation of oxytocinergic neurons in the PVN and leads to an elevation of OT spinal content, which may account for a tonic antihyperalgesic effect. In lamina II of the spinal cord, OT receptor activation seems to involve the recruitment of ERK1/2 and the stimulation of the local synthesis of allopregnanolone, which in turn increase $GABA_A$ receptor-mediated inhibitory tone.

Acute OT control of spinal nociceptive processes and pain responses is well documented and several mechanisms have been identified to explain these effects. In previous studies, endogenous OT release in the spinal cord was triggered by an electrical stimulation of PVN neurons (Martínez-Lorenzana et al., 2008) and produced a massive and sustained reduction in action potential firing in dorsal horn neurons (Yirmiya et al., 1990; Shiraishi et al., 1995; Condés-Lara et al., 2006). This reduction is fully prevented by selective OT receptor antagonist and affects selectively nociceptive inputs carried by Aδ and C fibers (Condés-Lara et al.,

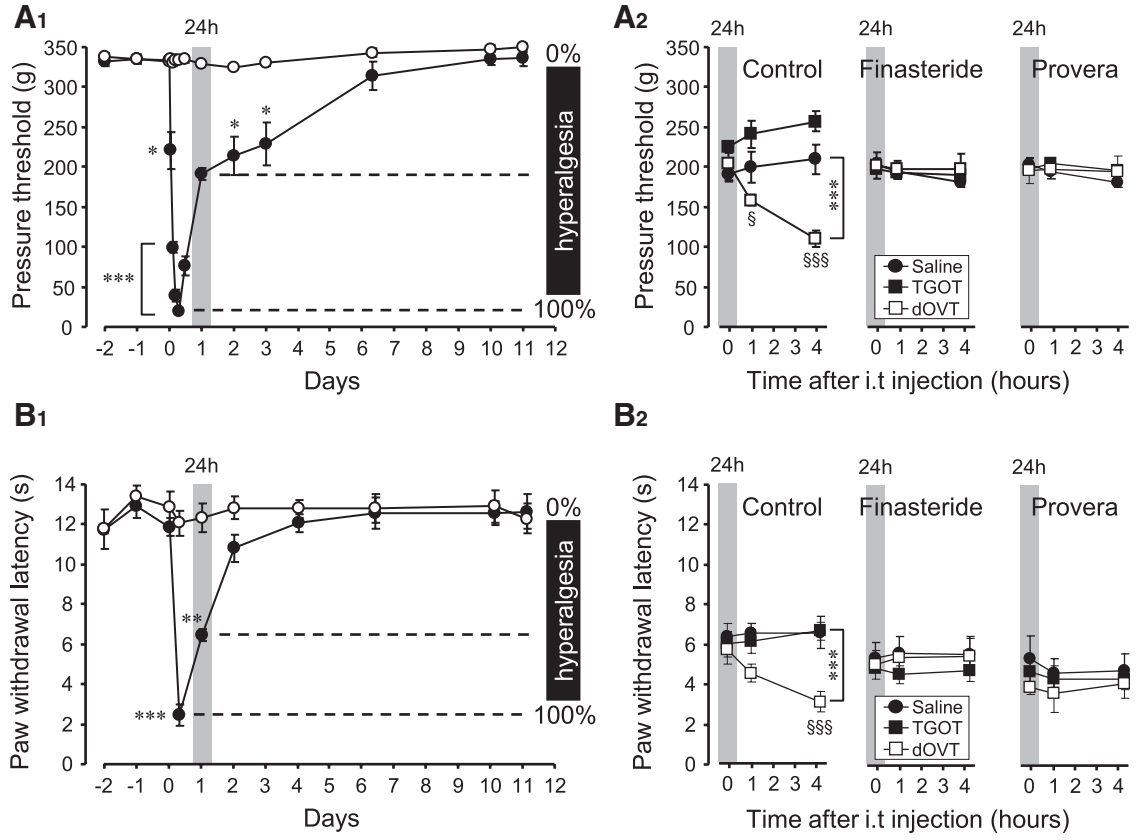

Figure 3. Limitation of mechanical and thermal hyperalgesia in carrageenan-injected rats resulting from the stimulation 3α5α neurosteroid synthesis by endogenous oxytocin. ***A1***, ***B1***, Time course of mechanical (pressure, ***A1***) and thermal (heat, ***B1***) nociceptive thresholds measured from the right (carrageenan-injected, black circle) hindpaw and the left (noninjected, open circles) hindpaw. The gray bar indicates values obtained 24 h after the carrageenan injection, which will be used as controls for the subsequent intrathecal injections (***A2*** and ***B2***). Two-way ANOVA (repeated measures; time × side) revealed a significant interaction for pressure and latency thresholds. Statistical differences are indicated after Bonferroni *post hoc* tests while comparing the thresholds between side for a given time point (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). ***A2***, ***B2***, Mechanical and thermal nociceptive thresholds changes in carrageenan-injected hyperalgesic rats (24 h, gray bar), assessed before (0 h) and after (1 and 4 h) intrathecal injection of saline, TGOT (100 nM), or dOVT (1 μM). Three groups of rats were used and pretreated 1 week before carrageenan injection with subcutaneous vehicle (control, left graphs), finasteride (30 mg/kg, middle graphs), or Depo-Provera (20 mg/kg, right graphs). Note that dOVT-induced hyperalgesia (seen in control condition) was never observed in finasteride or Provera-pretreated animals. Two-way ANOVA (repeated measures, time × treatment) revealed a significant interaction only in the control condition for pressure and latency thresholds. Statistical differences are indicated after Bonferroni *post hoc* tests while comparing the thresholds between groups for a given time point (***$p < 0.001$ compared with saline) and within time for each group ($^{\S}p < 0.05$; $^{\S\S\S}p < 0.001$ compared with time 0).

2006; Miranda-Cardenas et al., 2006). Many lines of evidence indicate that acute spinal OT analgesia is associated with a reinforcement of GABA receptor-mediated inhibition. In a recent study, we proposed that oxytocinergic control is relayed by a subset of glutamatergic neurons in lamina II, which recruit most, if not all, GABAergic interneurons (Breton et al., 2008). This hypothesis is supported by the elevated frequency of spontaneously occurring $GABA_A$ IPSCs, but not of miniature events. Whether this increase in $GABA_A$ receptor-mediated synaptic transmission is responsible for the inhibition of glutamatergic sensorispinal synapses (presynaptic inhibition) and/or of second-order spinal neuron integration is still unclear (Robinson et al., 2002). In all cases, OT action results in a strong reduction of spinal pain processing and this might also involve changes in the integration properties of dorsal horn interneurons, as previously suggested (Breton et al., 2009).

Here, we demonstrate that OT receptors are not only tonically activated in inflammatory pain states but also inhibit spinal nociceptive processing (Fig. 1*B*) and exert significant antihyperalgesia in carrageenan-induced pain model (Fig. 3). As shown by the high number of OT neurons immunoreactive for FosB/ΔFosB, the elevated OT content in lumbar vertebral segments of the spinal cord is likely to result from a sustained activation of hypothalamospinal oxytocinergic axons. It is interesting to note that the levels of OT in the blood, the CSF, and sometimes CNS tissue (spinal cord and encephalon), are directly related to the intensity of pain symptoms. Indeed, children exhibiting recurrent abdominal pain show reduced blood OT concentration (Alfvén et al., 1994), as well as in adult patients with low back pain (Yang, 1994). Recently, OT content was measured in the blood, CSF, and lumbar spinal tissues collected from neuropathic rats (Martínez-Lorenzana et al., 2008). OT concentration in the blood and CSF of neuropathic rats was significantly lower compared with control rats, whereas no change was observed in lumbar spinal tissues. In the carrageenan model of inflammatory pain, we found that spinal OT contents are indeed elevated and this difference is likely to be related to the inflammatory nature of the painful stimulus and/or the moment where tissues were collected and analyzed after pain induction. In good agreement, OT content in the CSF is increased 24 h after a sciatic nerve loose ligation and returned to control (pain free) values, 8 d later (Martínez-Lorenzana et al., 2008). Unfortunately, there is no similar time course for spinal OT available in the literature.

There is very little (and sometimes contradictory) information regarding the intracellular molecular mechanisms recruited by central OT receptor activation. Several reports are pointing

out the possible major contribution of mitogen-activated protein kinases (MAPKs) in the control of pathological pain processes (Ji et al., 2009). In line with this idea, anxiolysis produced by hypothalamic infusion of OT was recently demonstrated to result from the activation of ERK, a preeminent member of the MAPK family (Blume et al., 2008). ERK activation in pain conditions might promote long-lasting genomic changes but was also demonstrated to change spinal neuron excitability (Hu and Gereau, 2003; Hu et al., 2003). In this study, we provide preliminary evidence for a novel ERK-driven mechanism of analgesia, which involves the stimulation of neurosteroidogenesis, more specifically the synthesis of the well known analgesic neurosteroid allopregnanolone (Charlet et al., 2008). Interestingly, only the most superficial layers and particularly the neurons in lamina II are capable of producing allopregnanolone from cholesterol (Inquimbert et al., 2008). Since the neurosteroid-mediated long-lasting OT analgesia affects only C-type nociceptive messages, we can hypothesize that the increase of inhibitory tone is located in this lamina highly innervated by C nociceptors. As observed in Figure 3, we failed to observe any additional analgesic effect following TGOT administration. This leads us to suggest that 24 h following induction of inflammation the neurosteroid synthesis has reached its maximum. In a previously published paper (Poisbeau et al., 2005), acute inhibition of neurosteroidogenesis by finasteride (single injection 24 h before carrageenan injection) was associated with a significant decrease of thermal hot but not of mechanical nociceptive threshold. In this study, finasteride treatment was without effect. Without knowing the underlying mechanisms, we can only speculate that this might be due to homeostatic compensation resulting from the repeated exposure to finasteride (three injections every 2 d). How ERK activation can stimulate neurosteroidogenesis in the CNS is still unknown. The only available data refer to a possible steroidogenic role of ERK in peripheral gonadic cells (Cameron et al., 1996; Das et al., 1996; Gyles et al., 2001; Martinelle et al., 2004). Recently, a functional interaction between ERK and the TSPO complex was suggested (Poderoso et al., 2008, 2009). The contribution of ERK could possibly be mediated by its binding to the steroidogenic associated receptor protein, a major component of TSPO complexes (cholesterol translocator protein complexes). This working hypothesis remains to be demonstrated in the spinal cord and will require further investigations.

In summary, this study provides evidence indicating that increased release of OT by hypothalamospinal axons in hyperalgesic rats, tonically activates lamina II dorsal horn spinal OT receptors and, at least, the downstream intracellular effector ERK1/2. This mechanism leads to the stimulation of the synthesis of analgesic neurosteroids. We demonstrate this novel mechanism to be responsible for a sustained analgesic control in this particular inflammatory pain model. Analgesia is apparently achieved, at least, by an increase in the quantal inhibitory charge carried by synaptic $GABA_A$ receptors in lamina II neurons, via an allosteric potentiation by allopregnanolone-like neurosteroids.

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